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THE WALTER AND ELIZA HALL INSTITUTE OF RESEARCH IN PATHOLOGY AND MEDICINE

THE DIRECTOR'S TWENTY-FIRST ANNUAL REPORT

1939-40

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Historical Preface

The Walter and Eliza Hall Institute of Research in Pathology and Medicine was founded in 1916. The Trustees of the late Walter and Eliza Hall completed the Pathological Block of the present Royal Melbourne Hospital, and provided an annual payment of £2,500 towards the upkeep of the Institute. In 1925 this was increased to £3,100, and since 1930 has been £3,200 annually. In addition, the Institute has received, since its inception, £500 per annum from the University, the Medical Staff of the Melbourne Hospital having agreed that this portion of its clinical fund should be so used. Since 1925 a further sum of £250 per annum has been provided by the University from interest on the Appeal Fund (1924), together with an annual contribution from the Clinical Research Fund.

In 1926 the late Sir Aaron Danks, then President of the Melbourne Hospital, established an Endowment Fund to support a Biochemical Department. This fund has now reached the total of £8,612. The Edward Wilson Trustees maintained this new department by contributing £1,500 per annum in 1926, 1927 and 1928, and £1,000 in 1929. This Trust also, in 1926, contributed £3,000 for the foundation of an Institute Library, and of this sum £2,000 has been invested as endowment.

The Institute has been supported by annual contributions from the Felton Bequest Committee (since 1928), the Trustees of the late Anthony Mackie (since 1933), and the Trustees of the late T. J. Sumner (since 1929). The Trustees of the late Alfred Edments have also helped us since 1937.

The Institute has received substantial support from the Department of Health of the Commonwealth Government; in the years 1927, 1928, 1929, 1930 and 1931 a total of £10,650 was received for special researches. In 1934 the Commonwealth Government entered into an agreement with the Rockefeller Foundation that each would contribute to the Institute the sum of £1,000 per annum for the ensuing three years to support work on Virus diseases, particularly those affecting the central nervous system. In 1937 the Rockefeller Foundation and the Department of Health of the Commonwealth Government agreed to continue their support of this work for a further two years. The National Health and Medical Research Council have provided additional funds during 1938, 1939 and 1940, and Mr. E. Alex. Cato has generously arranged to contribute £1000 per annum for the five years' period from 1st January, 1939, to 31st December, 1944.

In 1932, by the will of the late Mrs. L. E. W. Carty, of Brisbane Hill, Hamilton, the Elsie Marion Carty Fund was founded. Mrs. Carty willed to the Institute a portion of her estate and a residuary interest therein to establish this Fund. It was her wish that the income should be used, primarily, for the assistance of promising research workers.

From 1934 to 1937 Mr. A. M. Nicholas gave an annual sum of £250 towards the salary of the Assistant Director, and Mrs. Nicholas generously continued this contribution in 1938 and 1939.

In 1936 the Carnegie Corporation of New York paid the travelling expenses of Dr. W. Feldberg and his family to Australia, and contributed in addition 4,500 dollars, which, with £1,100 collected by Mr. Reuben Hallenstein, made it possible to maintain this worker for two years.

The establishment of the National Health and Medical Research Council in 1937 has aided greatly in the development of this and other institutes for medical research in Australia.

The Institute is controlled by a Board consisting of representatives of the Walter and Eliza Hall Trust, of the University of Melbourne, and of the Committee of Management and Medical Staff of the Royal Melbourne Hospital. During the war years Professor Sir Harry Allen acted as Honorary Director. In 1919 Dr. S. W. Patterson was appointed first Director; in April, 1923, he resigned, and was succeeded by Dr. C. H. Kellaway in August, 1923. During the interval Mr. H. Dew acted as Director.

The Director's Twenty-First Annual Report

TO THE BOARD

OF THE

Walter and Eliza Hall Institute of Research in Pathology and Medicine

JULY, 1940.

The decision to rebuild the Royal Melbourne Hospital on the new site adjacent to the University at Parkville is at last being put into effect. The Committee of Management of the Royal Melbourne Hospital has accepted the responsibility for a new building for the Institute which will cost about £45,000. Towards this amount a generous gift of £20,000 has been made by Mr. G. R. Nicholas and by the family of the late Mr. A. M. Nicholas—Mrs. A. M. Nicholas, Mr. Maurice Nicholas, and Mrs. William Gluth. Mr. Russell Grimwade has given £1000, the Broken Hill Proprietary Company Ltd. £1000, Mrs. A. E. Ramsay £50, and Miss Mavis Freeman and Miss Dorothy D. Faris each £5. Mr. Basil Kilvington has contributed £21 towards the endowment of the Institute.

The new Institute, which is already well on the way towards erection, will provide much-needed additional laboratory space and greatly improved facilities. It will also bring us into more intimate relationship with the University, and it is hoped that the day is not far distant when a new Medical School will also be built on this site.

The war has had a considerable effect upon the Institute, and it is realised that we shall have much difficulty both in staffing and maintaining it during the next few years. Dr. Ian Wood, who resigned his Marion Carty Fellowship at the end of last year, Dr. A. V. Jackson, who had a junior fellowship of the National Health and Medical Research Council in the department of Virus Research, Dr. L. E. Rothstadt, our honorary heart specialist, and Mr. Claude Thomson, who was a technician in our media department, have gone overseas with the A.I.F. Miss Mavis Freeman, from the Virus Department, has enlisted as a V.A.D. assistant to Dr. E. V. Keogh, of the Commonwealth Health Services, who worked in Dr. Burnet's department from 1934 to 1937, and who has taken charge of the pathological laboratory of a general hospital on service abroad. Dr. G. V. Rudd is taking charge of the pathological department of another general hospital. Dr. Stanley Williams, who for several years was associated with the work of the Virus Department, has also enlisted. Miss Dora Lush left us in November to go to England to work under the direction of the late Sir Patrick Laidlaw at the National Institute for Medical Research.

During the war the policy of the Institute is being directed towards assisting the Defence Department in every possible way. In August last I joined the Medical Directorate at Army Headquarters in a part-time capacity. Later in this report some mention is made of various war activities of the members of the Institute staff, particularly in connection with the development of blood transfusion technique for use in the Navy, Army, and Air Force, in blood grouping nearly 13,000 members of the A.I.F. and in inoculating a number of doctors and nurses who were going overseas. Though as yet the general researches of the Institute have suffered but little interruption, our attention is now being concentrated on problems the solution of which appears to be important in the present struggle. Dr. Burnet, for example, is preparing attenuated influenza virus for use should there be a large scale epidemic of influenza; bacteriophages are being prepared for the laboratory diagnosis of dysentery, and the problem of the use of serum for the treatment of shock and haemorrhage is being explored.

A new departure has been made this year in commencing the publication of a series of Institute monographs. We have been particularly fortunate in securing the invaluable help of Dr. Colin W. Ross, who in an honorary capacity has undertaken the editorship of the series. Messrs. MacMillan and Co. have agreed to be responsible for their distribution. The first of the series is by Dr. F. M. Burnet, and is entitled "The Production of Antibodies: A Review and a Theoretical Discussion"; the second is by Dr. E. Graeme Robertson, the Neurological Research Fellow, and is devoted to a study of encephalography, and the third is by a number of authors, and describes the technique for blood transfusion for the Australian Navy, Army, and Air Force.

The work of the Institute has received further recognition this year by my election as a Fellow of the Royal Society. Several members of our staff are to be congratulated upon having gained their M.Sc. this year: Mr. D. E. Bland, with first class final honours and the exhibition, Miss Beryl Splatt, and Miss Marjorie Bick.

The Institute has had two guests this year, both working in the Virus Department: Miss Dorothy D. Faris, assistant Professor of Bacteriology in the southern branch of the University of Idaho, and Dr. S. E. Williams, who has been appointed research officer in charge of the newly founded Virus Department of the Commonwealth Serum Laboratories at Broadmeadows.

VIRUS DEPARTMENT.

Influenza Virus Studies.

I. *The Victorian Epidemic of Influenza in* 1939.—In June and July, 1939, a typical epidemic of influenza involved most of Victoria, and a number of investigations were carried out on the virus strains responsible and on the serological reactions of patients and contacts. Four typical strains of influenza virus were isolated by ferret inoculation and adapted to mouse passage. These strains were studied immunologically by the usual methods, and were shown to fall into two groups, each containing two strains. One group was practically identical with our standard ''Melbourne'' strain, isolated in 1935; the other was of more specific character, at least when first isolated. Evidence was obtained that after mouse passage one of these specific strains developed less specific ''intermediate'' characteristics. The existence of a third group was deduced from analysis of the serum reactions of a young spontaneously immune ferret which had apparently been naturally infected during the epidemic. The types of virus encountered appear to be entirely comparable to those isolated in the English epidemic of 1937, and the results confirm the English finding that in what appears to be a homogeneous epidemic distinct types of virus can be isolated.

Work on the serological reactions of patients and contacts was confined to two populations, the Institute staff and the inmates of a ward in a mental hospital. In all instances persons with clinical influenza showed a sharp rise in neutralising antibody, but there was a marked difference in the proportion of subclinical infections in the two populations—few amongst the Institute staff, and a very high proportion in the mental hospital group. There was very little evidence in either group that low initial antibody rendered an individual more likely to suffer clinical (or subclinical) infection than if he possessed a higher titre. A comparative study of complement-fixing and virus-neutralising antibody showed a certain general parallelism, but individual sera showed gross discrepancies, thus confirming the work of others that the antibodies involved in the two reactions were quite distinct. Also in confirmation of work elsewhere, it was shown that swine influenza antibody titre was sharply increased after an attack of influenza.

II. *The Growth of Unadapted Influenza Virus in the Chick Embryo.*—Workers in Goodpasture's laboratory have described a technique for the inoculation of viruses and bacteria into the amniotic cavity, but have not yet reported work with influenza virus. We have applied this technique to the study of unadapted influenza virus strains which produce no lesions on the chorioallantois, and have obtained some interesting and potentially important results. All the influenza strains available, including first-passage ferret material from the 1939 epidemic, mouse-adapted strains, and strains which produce lesions on the chorioallantois, give rise to specific lung infections of the embryo. The respiratory epithelium is damaged and eventually cast off, giving finally complete disorganisation of the lung with a characteristic histological picture. Virus is present in abundance in the lung and in the amniotic fluid, and passage may be continued by this method indefinitely. A very useful index as to whether infection has occurred is provided by smears of the fluid in the trachea. An uninfected embryo shows only a few red blood cells and an occasional flake of normal epithelium. Influenzal infections give turbid fluids with characteristically damaged epithelial cells and leucocytes. The technique is somewhat more complicated and time-consuming than the standard chorioallantoic method, but is rapidly being standardised, and promises to be a very useful weapon for influenza virus research.

In smears of tracheal fluid from infected embryos the desquamated epithelial cells are highly vacuolated. Many of the vacuoles contain acidophilic material whose texture and arrangement suggest strongly that it can be regarded as accumulation of virus particles: in other words, these are true cytoplasmic inclusions.

III. *A Virucidal Substance in Human Nasal Secretion.*—In some experiments on human beings it was found that the egg-adapted influenza virus strains "W.S." and "Melbourne" could be administered intranasally to two individuals with low serum antibody, without inducing either infection or an antibody response. In an attempt to detect local antibody formation, tests were made of the serous secretion which can be collected by plugging the nostrils with a roll of gauze. Dr. Burnet and Miss Lush found that this fluid had a well-marked inactivating effect on influenza virus, but the action was of quite a different character from that of specific antibody. It progressed slowly with time, and the active agent, which has been referred to as V.I.A.

(virus-inactivating agent), appears to be an enzyme resembling lysozyme. The action is not specific for influenza virus, but can be shown equally well against herpes, B., and louping-ill viruses, and to a less extent against poliomyelitis virus. A number of other viruses were tested, with negative results.

So far little progress has been made in elucidating the significance of this agent. There is a moderately wide range in the activity of fluid from different individuals, and the level appears to remain approximately constant for each person, but we can find no correlation with susceptibility or insusceptibility to infections with respiratory viruses. Attempts to define the chemical character of V.I.A. have been fruitless; it is not easy to obtain sufficiently large amounts of nasal secretion, and a number of technical difficulties have been encountered in trying to titrate the activity of chemically treated material.

IV. *The Possibility of Checking Pandemic Influenza by Living Virus Vaccines.*—In a paper read at the meeting of the Royal Australian College of Physicians, Dr. Burnet suggested that there was more than a normal likelihood that a pandemic of influenza would arise in Europe in one or other of the next two European winters, when a normal epidemic peak would be associated with war conditions. In discussing the possibility of using immunological measures for the partial control of a pandemic he considered that only by the use of living, partly attenuated virus given by the natural route was successful immunisation likely to be achieved. The chorioallantoic and amniotic methods of chick embryo inoculation make it practicable (a) to develop and maintain strains of virus whose virulence can be adjusted (for the ferret, and presumably for man) to any desired level, and (b) to produce large amounts of virus of standardised virulence at short notice, fertile eggs being the only experimental animal which is regularly available in almost unlimited numbers. Work is in progress to obtain and characterise a set of influenza virus strains of graded virulence which could serve as "seed virus" for rapid vaccine production in such an emergency. Attention is also being paid to the various technical aspects of producing and administering a living vaccine of this type.

The Action of Some Surface-Active Agents on Viruses.

While the work on V.I.A. was in progress, Wilson Smith's paper on the selective action of sodium desoxycholate on influenza virus appeared. It was of interest that the viruses sensitive to V.I.A. seemed to be also those which were easily inactivated by sodium desoxycholate. After testing a number of surface-active agents suggested by Dr. I. W. Wark, a detailed study of the activity of three—sodium desoxycholate, sodium lauryl sulphate, and saponin—was made. Ten different viruses were studied, and it was found that they could be arranged in a single order of decreasing susceptibility to all three agents, herpes virus being the most sensitive, and psittacosis virus the least. Sodium desoxycholate and sodium lauryl sulphate show a gradual quantitative change in susceptibility from one end of the series to the other, but the action of saponin is much more sharply differential, the viruses being divisible into two groups, one highly susceptible, the other almost completely insusceptible. It is of great interest that the saponin sensitive viruses are, with one exception, those which are susceptible to V.I.A., and also are those which are strongly inactivated by homologous immune sera.

The activity of the three chemical agents on a highly susceptible virus such as herpes is almost the same as their haemolytic power for rabbit erythrocytes, suggesting that the mechanism responsible for haemolysis and for virus inactivation is of similar character.

Psittacosis virus and all the bacteriophages tested were completely resistant to the action of these agents. It has been suggested that treatment of sputum from suspected cases of psittacosis with sodium lauryl sulphate (0.1 per cent.) should inactivate any pneumococci present without influencing the virus. This should make it possible to isolate the virus by mouse inoculation from a larger proportion of cases. No opportunity has yet arisen to test the method in practice.

Poliomyelitis.

In the last Annual Report an account was given of work on poliomyelitis arising out of the epidemic of 1937-38. Papers dealing with these studies have now been published, and a small amount of further experimental work has been carried out during the year covered by this report.

These have been concerned chiefly with the greater ease with which spread along nerves takes place in cynomolgus monkeys. Intradermal infection is much more readily produced than in rhesus monkeys, and in a fair proportion of cases virus can be isolated from peripheral nerves.

Dr. Burnet has published a general summary of the experimental work, and a discussion of its possible implications for the epidemiology of poliomyelitis. In this paper an attempt has been made to offer an explanation of two important points in the epidemiology of the disease, which have been rather neglected in previous writings: the sharp change in age incidence in the last thirty or forty years, and the occurrence of poliomyelitis epidemics almost exclusively in those countries which have the highest standard of living. It is suggested that poliomyelitis in human beings cannot be regarded as a strictly or even predominantly nervous disease, but rather as essentially a superficial pharyngeal infection which, like most superficial mucous membrane infections, does not produce any lasting immunity. The change in age incidence which has changed the disease from the strictly "infantile paralysis" of the early years of the century to one with its peak in early school life is thought to be due to a change in the character of the virus, rendering it less capable of producing inapparent adult infections. Instead of being mainly a disease of adults, from whom infants were infected in paralytic form, it is now a disease transferred mainly from child to child. It is thought that this change could take place most readily in countries with a steadily improving standard of living and of personal hygiene.

Psittacosis.

An interesting human case of psittacosis was investigated during the year. The virus was isolated from sputum, and all the budgerigars in the patient's aviary killed and examined. Two had been bought a fortnight before the onset of illness, but, like the others, showed no evidence of infection. It was concluded that the infection could only have been acquired during the patient's stay of a few minutes in the bird shop where the budgerigars were purchased. The incident provides another reason for advocating at least some control of the cage-bird trade in Australia.

In the course of work on this strain of psittacosis virus it was found that intranasal inoculation of mice was a useful experimental procedure, the lesions developing rapidly, and large numbers of L.C.L. bodies being present in the consolidated areas. The most interesting feature was the production of discrete focal lesions when the inoculum was highly diluted. Counts of these lesions showed an approximately linear relation between their number and the concentration of virus administered. Dr. Rudd has applied this method to immunological and chemotherapeutic studies. The results were strikingly negative; convalescent human and hyperimmunised guinea-pig serum mixed with virus produced no reduction whatever in the number of foci, and several methods of attempted active immunisation of mice by living or killed virus equally failed to reduce the number of foci below that produced by the same test dose in normal mice. Chemotherapeutic tests with sulphanilamide, prontosil and sulphapyridine were completely negative.

A Modified Chorioallantoic Inoculation Technique.

The method of chorioallantoic titration of viruses used in this laboratory has remained unaltered for several years, but it has always been felt that there was room for improvement. non-specific ulcer type lesions and secondary colonies around the primary foci often making pock counts difficult. A simple means of overcoming these difficulties has been found. After the small slit in the shell membrane has been made a drop of normal saline (containing 0.044 per cent. $CaCl_2$) is placed over it. Suction is now applied by a teat over the opening in the air-space, and the drop of saline drawn into the potential space between shell membrane and chorioallantois. There it acts as a fluid wedge, allowing a much more gentle separation of chorioallantois from shell membrane than in the original technique. With practice, nearly 90 per cent. of "perfect" membranes, i.e., without non-specific lesions and with insignificant numbers of secondary foci, can be obtained.

Rickettsial Infections.

The most interesting development of the work on Q fever in the past year has been the recognition by Dyer that a rickettsial infection in ticks collected in Montana, U.S.A., was due to an organism immunologically identical with *R. burneti.* According to a personal communication, natural human infections with symptoms like those of Q fever have also been observed recently in Montana and shown to be due to this rickettsia. Dr. Dyer very kindly sent us an American strain, and Dr. Burnet and Miss Freeman have carried out a detailed comparison with the Australian type. This has fully confirmed Dyer's work, and indicates that immunologically the two strains are indistinguishable. The range of susceptible hosts is the same, but the American "X" strain is consistently more virulent for all hosts except mice. Q fever strains grow poorly on the chorioallantois. the X strain much more freely. A detailed study of the latter strain on the egg membrane has therefore been made, with some interesting results. Growth is very much better on younger embryos than on the standard twelve-day membranes. If eight- or nine-day embryos are inoculated and the membranes removed after six days incubation at 34° C. numerous rickettsiae are visible in smears and sections. They are chiefly present in the most superficial layer of ectodermal cells which are uniformly vacuolated, the vacuoles containing numerous rickettsiae. On twelve-day membranes the lesions contain relatively few rickettsiae, but with dilute material discrete foci develop, and such membranes can be used for pock counting titrations. Attempts to demonstrate the neutralising activity of immune sera by this method gave negative results. Serum which was known to have a high content of specific rickettsial agglutinins did not reduce the count of foci below that of the control.

After numerous passages on the egg membrane the X strain showed no reduction in virulence for the guinea-pig; egg virus, in fact, appeared to be considerably more virulent than either guinea-pig or mouse material. An analysis of this apparent greater virulence fully confirmed the fact, and indicated that the difference was not due to a greater number of rickettsiae in the egg material. Mouse spleen and egg membrane emulsions were prepared which, on the evidence of stained smears, contained approximately equal numbers of rickettsiae. Serial tenfold dilutions were inoculated into guinea-pigs. Egg virus produced fatal infections undiluted, and typical non-fatal reactions with high temperatures to a dilution of 10^{-7}. Mouse virus gave typical responses only with the first two dilutions: 1 : 100 virus gave only a moderate temperature rise, and the higher dilutions irregular rises only. Immunity tests, however, showed that all guinea-pigs injected with mouse virus to the 10^{-7} dilution were immune, and one of those given the 10^{-8} dilution, a result identical with that found with egg virus. The difference between the two types of virus recalls the well-known phenomenon of the much higher virulence of the rickettsia of Rocky Mountain spotted fever in ticks, as compared with guinea-pig virus, and in all probability the difference is due to the fact that in mouse or guinea-pig virus the rickettsiae are coated with antibody; this antibody coating is absent in egg or tick virus.

The higher virulence of the X strain for guinea-pigs has also allowed more clear-cut results to be obtained in regard to the immunity reactions of *R. burneti* in that species. One feature of interest concerns the production of fatal infections. Ordinarily transfer from guinea-pig to guinea-pig using spleen emulsions gives only a well-marked febrile reaction with recovery and occasional late deaths. If, however, an infection is initiated with a very large dose, especially of egg membrane virus, the animal dies in seven to twelve days, and shows *post mortem* enormous numbers of rickettsiae in smears of the splenic exudate and mesenteric lymph nodes. Subinoculation of this material allows a continued series of fatal infections. With smaller doses the incubation period lengthens and the severity of the febrile response diminishes. Subclinical infections occur with small doses, particularly of mouse virus, less often with guinea-pig virus from fatal infections, and not at all with egg virus. When any form of infection, clinical or subclinical, occurs, resistance to a subsequent injection is almost absolute. With very large test doses a proportion of immune animals show an "accelerated response" in the form of an immediate rise of temperature lasting two or three days; most show no reaction whatever.

As with other rickettsial infections, mixtures of virus and immune serum in suitable proportions may be injected without the appearance of a febrile response, but in all instances the subsequent complete immunity of the animal indicates that an actual infection has occurred. The same inhibition of symptoms can be seen when immune serum and virus are injected separately, though preliminary mixing is distinctly more effective. It seems that the symptom-provoking power of the rickettsiae is in some way greatly reduced when they are coated with antibody, although their capacity to induce infection is hardly affected.

Experiments with sodium lauryl sulphate, an agent which might be expected to produce a monomolecular film on the surface of the rickettsiae physically rather similar to an antibody coating, showed that mixtures of rickettsiae with solutions of sodium lauryl sulphate behaved on guinea-pig inoculation very like immune serum-rickettsia mixtures. Except with very small numbers of organisms, the rickettsiae were not killed, but their fever-producing capacity was almost annulled. It seems possible that the use of living rickettsiae partially inactivated by some surface-active agent of this sort might provide an effective means of vaccination against typhus.

Rickettsiae killed by heat or formalin produce partial immunity in guinea-pigs, the response to a large test dose being much smaller than in controls, but complete resistance is not readily produced.

The Production of Antibodies.

During the past few years a good deal of general immunological work has been carried out in this Department, particularly in regard to the site of production and the rate of disappearance of antibodies. Dr. Burnet has attempted to include the results of these experiments in a general review and discussion of the question of antibody production. Any such discussion leads at once to the general question of the synthesis of protein *in vivo,* and in the monograph this problem is approached much more from the biological than from the chemical standpoint. A very compressed account of the hypothesis of antibody production has been published as a preliminary note in the "Australian Journal of Science."

F. M. BURNET:

"Antibody Production as a Special Example of Protein Synthesis *in vivo,*" "Australian Journal of Science," 1939, 1, 172.

"The Epidemiology of Poliomyelitis, with Special Reference to the Victorian Epidemic of 1937-38," "Medical Journal of Australia," 1940, 1, 325.

"The Production of Antibodies: A Review and a Theoretical Discussion," 1940 (in the press).

"Influenza Virus Infections of the Chick Embryo Lung," 1940 (in the press).

F. M. Burnet, J. F. J. Cade, and D. Lush:

"The Serological Response to Influenza Virus Infection During an Epidemic, with Particular Reference to Subclinical Infection," "Medical Journal of Australia," 1940, 1, 397.

F. M. Burnet and M. Freeman:

"A Comparative Study of Rickettsial Strains from an Infection of Ticks in Montana (United States of America) and from Q Fever," "Medical Journal of Australia," 1939, 2, 887.

F. M. Burnet, M. Freeman, E. H. Derrick, and D. J. W. Smith:

"The Search for Immunological Relationship Between Q Fever and Other Rickettsioses," "Medical Journal of Australia," 1939, 2, 51.

F. M. Burnet, M. Freeman, A. V. Jackson, and D. Lush:

"A Study of Heterologous Antibodies in the Serum of Poliomyelitis Patients," "Medical Journal of Australia," 1939, 2, 198.

F. M. Burnet and A. V. Jackson:

"Poliomyelitis: 2. The Significance of Neutralising Antibodies in Human Sera," "Australian Journal of Experimental Biology and Medical Science," 1939, 17, 261.

F. M. Burnet, A. V. Jackson, and E. G. Robertson:

"Poliomyelitis: 1. Intraocular Inoculation as a Standard Method for the Demonstration of Neutralising Antibodies,"

"Australian Journal of Experimental Biology and Medical Science," 1939, 17, 253.

"Poliomyelitis: 3. The Use of Macacus cynomolgus as an Experimental Animal," "Australian Journal of Experimental Biology and Medical Science," 1939, 17, 375.

F. M. Burnet and D. Lush:

"Influenza Virus Strains Isolated from the Melbourne 1939 Epidemic," "Australian Journal of Experimental Biology and Medical Science," 1940, 18, 49.

F. M. Burnet, D. Lush, and A. V. Jackson:

"A Virus-inactivating Agent from Human Nasal Secretion," "British Journal of Experimental Pathology," 1939, 20, 377.

Miscellaneous Writings.

The following papers, etc., are not directly related to the current experimental work of the Department, but include incidentally some of the results of past work:

> "Biological Aspects of Infectious Disease," F. M. Burnet. This semi-popular book is being published in England by the Cambridge University Press and in America by the Macmillan Company.
>
> "Changes of Twenty-five Years in the Outlook on Infectious Disease," F. M. Burnet, "Medical Journal of Australia," 1939, 2, p. 23, a general account written for the 25th anniversary number of the "Medical Journal of Australia."
>
> "Viruses as Agents of Human Disease," in "Oxford Medicine," New York (in the press).
> "Q Fever," F. M. Burnet and E. H. Derrick, in the "British Encyclopaedia of Medical Practice," Supplementary Volume (in the press).
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> "Psittacosis in Australia," F. M. Burnet, and "Q Fever," F. M. Burnet and E. H. Derrick—two papers contributed to symposia at the Sixth Pacific Science Congress, 1939, at San Francisco.

Department of Physiology.

Dr. Trethewie and I have continued our work on the effects of tissue injury, and, apart from a few further experiments on anaphylaxis and on snake venom, have spent almost the whole of the period under review in the study of the toxins of the anaerobes. We have completed the investigation of only one of these, the toxin of Cl. welchii, type D, in which work we have collaborated with Dr. A. W. Turner, of the Division of Animal Health and Nutrition of the Council of Scientific and Industrial Research. Study of the toxins of Cl. welchii, Cl. septique, and Cl. oedematiens showed that their behaviour was different from that of the poisons and toxins whose injurious effects on the tissues had hitherto been examined in this laboratory by Feldberg, Keogh, O'Connor, Holden, Trethewie, and myself. The cardiac action of these toxins suggested that active substances other than those shown to be formed or liberated in injury by snake venoms, by peptone, by staphylococcal toxin, in anaphylaxis, by anaesthetics, by radiant energy, and by the salts of heavy metals, might be set free. The experiments of Drury and his colleagues, who had shown that when the perfused heart of the guinea-pig is injured by heat adenyl compounds are liberated and can exert their effects upon a second normal perfused heart, led us to investigate the possibility that other forms of injury may also cause the liberation of adenyl compounds, and that some of the symptoms caused by poisons and toxins may thus be explained. We made a preliminary study of the effects of cobra venom before continuing our work on the toxins of the anaerobes.

The Liberation of Adenyl Compounds by Cobra Venom.

The experiments of Drury, Lutwak-Mann and Solandt (1938) had provided a sensitive biological method for the estimation of adenyl compounds by their depressant effect on the contractions of the right auricle of an atropinised guinea-pig, the injections being made by a cannula in the left auricle, and so into the coronary circulation. In our experiments the depressant action of snake venom present in extracts and perfusates was eliminated by protecting the guinea-pig with antivenine, and we were able to show that lysocithin, which also has a cardio-depressant action, and which is formed by the venom, was not present in amount sufficient to affect the titration. Since neither adenosine nor adenylic acid was available, we used lacarnol (Bayer) as a standard in these experiments. We studied the effect of cobra venom on the rabbit's liver, which contains only traces of histamine, but is rich in adenylic acid. When a lobe of this organ was perfused with Tyrode solution no trace of adenyl compounds could be detected in the perfusate and no loss was detected by comparison of an extract of the perfused liver with that of a piece of the same liver which was subjected only to a short perfusion to remove blood. The injection of cobra venom caused a striking loss of activity in the extract after perfusion, but only a small proportion of this activity was estimated in the perfusate. By collection of the perfusate at 95° C. the proportion of adenyl compounds estimated in it after the injection of venom was increased, and this effect was shown to be significant by collection of perfusate in equal fractions one at room temperature and one at 95° C.

It appeared likely that cobra venom caused not only the liberation of active adenyl compounds, but also of an inactivating enzyme, and that much of the observed inactivation took place in the organ itself. Evidence in support of these probabilities was obtained by showing that lacarnol added to unboiled perfusate from an envenomed organ lost its activity, but that no inactivation took place in contact with perfusate from a normal organ, and that perfusion and reperfusion of a solution of lacarnol through a piece of normal liver caused rapid inactivation of the perfusate without any increase occurring in the activity of extracts of the perfused organ. We were able to inhibit inactivation in the perfused organ with cyanide. When a piece of liver was perfused with M/40 NaCN in Tyrode solution brought to pH 7.4 by the addition of HCl there was no appreciable loss of active substances from the organ, and when cobra venom was injected the amount of cardio-depressant substances liberated in the perfusate was approximately equivalent to the loss from the liver as estimated by difference of organ extracts.

Electrocardiographic studies in various species showed that changes in conduction occurred in the heart poisoned by cobra venom. These could not be explained by the liberation of histamine, but might be attributable (a) to gross lesions in the conducting tissues (which were never observed in these short experiments), (b) to direct injury by the venom itself or by lysocithin, (c) to changes in the blood supply of the bundle tissues, or (d) to the liberation of adenyl compounds. Now although the injection of adenyl compounds causes changes in conduction in some species, so great is the inactivating power of the blood and tissues that large doses are required to produce these effects. It is unlikely, therefore, that adenyl compounds liberated from organs other than the heart itself could give rise to cardiac effects. In experiments on the perfused heart of the cat we regularly found an output of adenyl compounds in the perfusate after the injection of cobra venom, but this did not occur if the venom was neutralised before injection with cobra anti-venine. Lysocithin was also shown to cause the liberation of adenyl compounds from these perfused organs.

These experiments support the view that some at least of the cardiac effects of this venom may be attributable to the liberation of adenyl compounds in the heart either directly by the venom itself or by lysocithin formed by its action upon the tissues.

The Circulatory and Neurotoxic Effects of Cl. welchii, Type D.

The toxin, purified by precipitation with ammonium sulphate, was shown in experiments on the isolated jejunum of the guinea-pig to contain at least two active principles, only one of which was neutralised specifically by antitoxin. The non-specific principle, which caused a histamine-like contraction of the gut and an immediate depressor effect on the blood pressure of the atropinised cat, was removed by precipitation with alcohol in the cold. This further purification was carried out for us by Mr. A. T. Dann, of the Division of Animal Health and Nutrition of the Council for Scientific and Industrial Research. It involved the loss of 84 p.c. of the weight of the dry toxin (precipitated by ammonium sulphate) and a decrease of the lethal dose for mice from 0.03 mg. to 0.008 mg. per 20 gm. The contraction of the isolated jejunum of the guinea-pig caused by this purified toxin had a short latency, and relaxation was relatively slow. Repeated administration of the toxin did not impair the response of the gut to histamine or to S.R.S., but the muscle became insensitive to the toxin itself. This "desensitisation" of smooth muscle was paralleled by the tolerance conferred upon animals by an initial sub-lethal dose of toxin, a second dose causing a smaller effect both on the blood pressure (vaso-constriction in the general and pulmonary circulations) and on the heart.

In lambs the toxin in a dose of from 0.1 to 0.13 mg. per kg. caused death in from two to six hours with dyspnoea, ataxia, loss of voluntary power in the muscles, convulsions, and failure of respiration. The convulsions could not be accounted for by hypoglycaemia, nor by hypocalcaemia, nor were they asphyxial in origin. The morbid changes observed post mortem were hydrothorax and hydropericardium, haemorrhages in the heart muscles and adrenals, and extensive haemorrhagic oedema of the lungs. There were no macroscopic nor microscopic changes in the brain or spinal cord, which were sectioned and examined for us by Dr. E. Graeme Robertson. The injection of toxin in these doses in lambs under chloralose anaesthesia and with the vagi cut were without any early effect on the blood pressure, but doses of from 0.2 to 1 mg. after a short latency caused a precipitous fall of systemic blood pressure, followed by a steep rise, with quickening of the heart beat. These early effects of large doses were shown to be attributable to still another non-specific impurity.

The action of the toxin on the nervous system of lambs was subjected to further analysis. Convulsive movements did not occur in the hind limbs after section of the spinal cord in the dorsal region. After decerebration, the brain stem being divided at a level immediately in front of the anterior colliculi, no convulsive movements of the facial muscles were observed and only irregularly spontaneous movements of the limbs. Infiltration of the motor cortex with local anaesthetic did not impair the contralateral convulsive movements. There was clinical evidence of depressant action on the tone of the limbs, but the toxin had no directly paralysant effect on the lower motor neurone.

Experiments in the rabbit showed that after doses ranging from 0.07 to 1 mg. per kg. death took place from cardiac failure and oedema of the lungs. With the larger doses, 1 mg. to 0.28 mg. per kg., there was temporary failure of respiration. In rabbits under chloralose with the vagi cut and artificial ventilation there was a sharp fall in systemic pressure, which was chiefly attributable to depressor action of the toxin on the heart. This was followed by a rise in systemic blood pressure which suggested an output of adrenalin and a final fall of blood pressure caused by cardiac failure. The toxin caused grave changes in conduction in the heart as shown by electrocardiograms.

In the cat the purified toxin caused death from acute pulmonary oedema, but it was not possible to analyse its pharmacological action because the doses (4 to 8 mg. per kg.), which caused severe early effects on the circulation, contained too much of the cardio-depressant impurity, which caused similar effects in the sheep.

The Liberation of Histamine and of Adenyl Compounds by the Toxin of Cl. welchii, Type D.

The toxin causes the liberation of histamine from the perfused lung of the cat, but rather large doses and a slow rate of perfusion were necessary to show this, and the output did not compare with that obtained after the injection of small doses of snake venom. The purified toxin also causes the liberation of adenyl compounds and of inactivating enzymes from the perfused liver of the rabbit and from the perfused heart of the cat. The degree of injury caused by this toxin is not comparable to that caused by cobra venom, since rather large doses are required to demonstrate its effects in the liberation of active substances into the perfusate. In the experiments with the rabbit's liver, the apparent loss of adenyl compounds from the perfused organ was much greater than the actual loss, because much destruction of adenyl compounds took place in the cells of the perfused organ after injury by toxin. When toxin was injected into pieces of liver perfused with neutralised M/40 NaCN in Tyrode solution there was much less loss, as judged by difference of organ extracts, and the amount of adenyl compounds detected in the perfusate was greatly increased, so that almost the whole of the adenyl compounds lost from the organ were estimated in the perfusate. In these experiments adenosine (B.D.H.) was used as a standard.

The Liberation of a Slow-reacting Muscle-stimulating Substance in Anaphylaxis.

A preliminary description of this work was given in the last annual report, and though the final results were substantially the same as those already reported, some additional facts have been brought to light. It will be remembered that in experiments carried out in this laboratory (Feldberg and Kellaway, 1938; Feldberg, Holden and Kellaway, 1938; and Trethewie 1939), it was shown that the contractions of smooth muscle caused by some snake venoms are largely determined by the formation of a slow-reacting muscle-stimulating substance. Our studies on anaphylaxis were made to discover whether a similar muscle-stimulating substance is formed or liberated in the anaphylactic reaction and whether the anaphylactic contraction of smooth muscle can be attributed to it.

The response of the isolated jejunum of the sensitised guinea-pig to the antigen was shown to resemble those caused by venom and by slow-reacting substance (S.R.S.) formed from venom in its latency, in the time required for relaxation and in the after-changes in the excitability of the muscle. Various states of depressed excitability in the isolated jejunum of the sensitised guinea-pig were brought about by the administration of S.R.S., lysocithin, histaminase, CO_2, the toxin of Cl. welchii, type A, and by photodynamic action and the contractions in response to antigen, to histamine, and to S.R.S. were compared under these conditions. The failure of the gut to respond to histamine after CO_2 had been bubbled through the bath was shown not to result from the inactivation of histamine by CO_2, but from a depressant effect of CO_2 on the muscle, causing a diminution in its response to various stimuli. In this and in most other conditions of depressed excitability the response to antigen, to histamine, and to S.R.S. were depressed to the same extent, but there were three exceptions. After repeated treatment with S.R.S., the response to S.R.S. was greatly reduced or abolished, but that to histamine was unimpaired, and that to the antigen only slightly affected. During recovery from poisoning with lysocithin the response to the antigen was only slightly reduced, while those to histamine and to S.R.S. were greatly reduced. After treatment with the toxin of Cl. welchii, type A, the response to S.R.S. was greatly reduced, whereas those to histamine and to the antigen were unimpaired. A similar dissociation was observed with the guinea-pig's uterus after lysocithin.

These results suggest that histamine, and not S.R.S., is responsible for the anaphylactic contraction of these smooth muscles and differentiate between the anaphylactic response of the gut and that caused by snake venoms because, after treatment with the toxin of Cl. welchii, type A, the gut fails to respond to an initial dose of venom. On the other hand, it has been shown that when the uterus of the guinea-pig is poisoned with large doses of histamine it reacts to further doses by relaxation, whereas S.R.S. and the anaphylactic antigen still cause contractions. We could not therefore exclude absolutely either histamine or S.R.S. as stimulant principles participating in the anaphylactic contraction of plain muscle, and the demonstration that in addition to histamine, a muscle-stimulating substance similar to that formed by the action of venom is liberated in the tissues of sensitised animals by the action of the antigen, still leaves open the possibility that both these substances are involved.

The increase of S.R.S. in fresh extracts of the perfused lung and intestine, after the arterial injection of antigen or after incubation with it, suggested that S.R.S. was formed during the antigen-antibody reaction, but extracts of dried tissues which gave a greater yield of S.R.S. from normal tissues than did fresh extracts showed no increase in S.R.S. after antigen had been allowed to act upon them. It is probable, therefore, that the yield of S.R.S. obtained by saline extraction of fresh tissues is incomplete, and that the increase after the action of antigen must be explained by liberation rather than by formation of S.R.S. It may be mentioned that in agreement with the conclusion of Feldberg and Kellaway that S.R.S. was formed by the venoms, desiccated extracts of tissue incubated with venom contained much more S.R.S. than control extracts. The amount of S.R.S. detected in extracts as a result of the interaction of antigen and antibody was so much less than that obtained by the action of venoms that the difference between the superficially similar S.R.S. set free in the two reactions may ultimately prove to be a qualitative one.

Mr. Basil Kilvington has continued his researches on rearrangement of the fibres in peripheral nerves during regeneration after injury. Unhappily, he will not be able to carry this on during the war because he is once again taking up work in the Hospital as an in-patient surgeon.

C. H. Kellaway and E. R. Trethewie:

"The Liberation of Adenyl Compounds from Perfused Organs by Cobra Venom," "The Australian Journal of Experimental Biology and Medical Science," 1940, 18, 63.

"The Liberation of a Slow-reacting Muscle-stimulating Substance in Anaphylaxis," "The Quarterly Journal of Experimental Physiology," 1940, 30, p. 121.

C. H. Kellaway, E. R. Trethewie and A. W. Turner:

"Neurotoxic and Circulatory Effects of the Toxin of Cl. welchii, Type D," "The Australian Journal of Experimental Biology and Medical Science" (in the press).

"The Liberation of Histamine and of Adenyl Compounds by the Toxins of Cl. welchii, Type D," "The Australian Journal of Experimental Biology and Medical Science" (in the press).

BIOCHEMICAL DEPARTMENT.

Haemocyanin.

Mr. Rawlinson has prepared a large number of batches of crystalline haemocyanin from the Australian crayfish (*Palinurus vulgaris*), and has determined some of its physical and chemical constants. The iso-electric point is at pH 4.6; the specific rotation using the mercury arc is—42.5° at pH 7.0 and 20° C. A diffuse spectroscopic band was observed at 5580 A; in the ultraviolet there are bands at 3350 A and 2780 A, the former related to oxygenation and the latter dependent upon the protein moiety of the haemocyanin molecule. The copper content is 0.148 p.c. The oxygen capacity determined with the Toepler pump is 25 c.c. per 100 gm. haemocyanin in comparison with haemoglobin, which has an oxygen capacity of 134 c.c. The prosthetic group, which was thought to have been isolated by earlier workers, probably consists of basic copper sulphide admixed with protein breakdown products. Unlike the blood pigment of mammals, haemocyanin does not combine with carbon monoxide. The ultra-violet spectrum of oxyhaemocyanin treated with carbon monoxide is that of reduced haemocyanin, whereas those of oxy- and of carbon monoxide haemoglobin are nearly the same. The absence of a carbon monoxide compound was confirmed by Mr. Bland, who found no more carbon monoxide present than was attributable to physical solution.

Magnetic Properties of Haemin.

Mr. Rawlinson has used the magnetic balance for a series of determinations on haemin dissolved in various solvents. In purified pyridine haemin has a low magnetic moment, and the fall from the value for solid haemin (5.9) is probably attributable to partial combination with the solvent, and not to decomposition changes. Pyridine parahaematin, which has a magnetic moment of 1.97, has one unpaired electron, the six covalent bonds of the iron atom being arranged octahedrally. The magnetic moment of haematin in sodium hydroxide solution (3.6) suggests the presence of an intermediate type of linkage with three unpaired electrons. This form of linkage is rarely met in iron compounds. The magnetic moment of haemin in alkaline 30 p.c. cane sugar solutions is 5.6, a value which approximates to that obtaining for compounds with ionic linkages (five unpaired electrons). This change in moment from 3.6 to 5.6 in the presence of strong cane sugar solution may be related to dehydration.

Determination of Traces of Carbon Monoxide in Blood and Air.

Mr. D. E. Bland studied the determination of minute amounts of carbon monoxide in small samples of blood and air. He combined the iodine pentoxide method for the determination of carbon monoxide with the catalytic determination of iodine as described by Kolthoff, and has developed this combined method so as to obtain consistent and accurate results. In normal blood the amount of carbon monoxide present was estimated in 1 c.c. samples, and it was found that about 1 p.c. of the blood pigment takes no part in the transport of oxygen owing to its combination with carbon monoxide. He has designed and constructed a compact apparatus suitable for research in industrial hygiene. The method can be used on samples of 0.2 c.c. from a finger prick.

For determinations of the carbon monoxide in air the method is most suitable because it requires much smaller samples than the standard methods.

Carbohydrate Metabolism of Yeast.

In September, 1939, Dr. A. Gottschalk joined the department. He has been studying the carbohydrate metabolism of the yeast cell under conditions involving the strict exclusion of all oxygen.

Preparation of Protoporphyrins and Metallo-porphyrins.

Mr. Holden has attempted the direct preparation of protoporphyrin from reduced blood with a view to eliminating the costly intermediate preparation of haemin. It was found, however, after a number of experiments, that the yields of crystalline product were too low to admit of any economy as compared with the standard method. A number of specimens of metalloporphyrins have been prepared for the study of their spectra in various solvents and when combined with proteins.

Slow-reacting Substance.

Mr. Holden has continued his studies on slow-reacting substance (S.R.S.) produced by the action of cobra venom on egg yolk. It has been found possible to shorten the preparation of a crude extract by extracting it from the first alcoholic solution by petroleum ether. A subsequent concentration was effected by solution in a limited amount of methyl alcohol, or better, of acetone. The use of the latter solvent in addition removes some extraneous substance that rapidly desensitises the test strip of isolated guinea-pig intestine. An oily product is obtained, of which about 0.1 mg. gives the characteristic contraction and after-effects on the sensitivity of the gut. The substance appears to be unsaponifiable and to have an acidic group. The active principle is destroyed by acids, and its calcium, barium, and lead salts do not assist in separating it. An attempt at distillation in a vacuum of 0.05 mm. resulted in some of the active substance distilling over, but much more was destroyed.

An Adjustable Sealed Mercury Thermo-regulator.

Mr. Rawlinson has devised a sealed thermo-regulator for use with a thermionic relay. In essentials this is a thermometer type of regulator with two fixed platinum contacts, and adjustment is possible by transferring mercury to a side reservoir.

Other Work.

Since the beginning of September, 1939, some time has been devoted to the assistance of various people in difficulties caused by the war. One that may be worth noting is the preparation from Australian commercial methanol of a methyl alcohol of sufficient purity to be used in Leishman's stain as a substitute for the German material hitherto employed.

David E. Bland:

> "A Micro Method for the Determination of Carbon Monoxide in Blood," "Australian Journal of Experimental Biology and Medical Science," 1940, 18, 35.

W. A. Rawlinson:

> "Crystalline Haemocyanin—Some Physical and Chemical Constants," "Australian Journal of Experimental Biology and Medical Science," 1940, 18, 131.
>
> "The Variation in the Magnetic Susceptibility of Haemin in Various Solvents," "Australian Journal of Experimental Biology and Medical Science" (in the press).
>
> "An Adjustable Sealed Mercury Thermo-regulator for Use with a Thermionic Relay," "Australian Journal of Science," 1939, 2, 30.

CLINICAL RESEARCH.

Dr. Ian Wood, who was Marion Carty Fellow until the end of last year, and Miss Bick have continued their studies on the biochemical changes associated with paralytic ileus and with severe haemorrhage. This work has been made possible by a grant from the National Health and Medical Research Council for Miss Bick's salary, and for the purchase of apparatus.

Paralytic Ileus.

Associated with the extensive loss of water, salts, and food from the upper portions of the alimentary tract, there is in this condition a deficiency of sodium chloride, some degree of acidosis, and a depletion of the plasma protein content of the blood. Treatment aims at the correction of these deficiencies. Large volumes of glucose saline are infused into the veins, and an intravenous infusion of hypertonic saline, Hartmann's sodium lactate solution or serum may have to be given, depending on the degree of the observed biochemical changes.

Haemorrhages from the Stomach and Duodenum.

The investigation of the biochemical changes occurring in the blood and urine as a result of severe haemorrhage from the stomach and duodenum shows that there is depletion of the blood volume, accompanied by a rise in the urea, and a fall in the alkali reserve, and in the protein and chloride content of the blood. The urine is diminished in amount and its chloride content greatly lowered. Dr. Wood and Miss Bick have studied the effects of treatment and the correction of these abnormalities by massive blood transfusion, by the intravenous infusion of saline and Hartmann's sodium lactate solution, and by Meulengracht's high caloric feeding. After the haemorrhage has ceased the blood urea falls from its high level, and this fall is hastened by the liberal administration of fluid. If the fluid intake is restricted, the excretion of urine is diminished and the blood urea remains high. The increased excretion of urea during recovery is related to the volume of urine secreted and the urea concentration is usually low in comparison with the height of the blood urea.

The Rise of the Blood Urea During Gastro-intestinal Haemorrhage.

Dr. E. B. Drevermann has attempted to determine the cause of this rise in blood urea by experiments upon himself and on some of his colleagues, the effects of gastro-intestinal haemorrhage being simulated by bleeding from a vein and introduction of the shed blood into the stomach. Preliminary experiments concerned the relation of the level of blood urea to the protein and fluid in the diet. It was shown that external haemorrhage could cause an increase in the urea of the blood by decreasing urinary excretion and that the introduction of blood into the stomach caused a rise in the blood urea because of the digestion, absorption and deamination of the blood proteins. Under the conditions of these experiments, it appeared that compensatory retention of fluid following haemorrhage into the stomach or duodenum and the absorption of blood proteins from the intestine were sufficient to account for the observed rise in the blood urea.

Blood Storage.

Miss Bick has continued her work on the changes observed during the storage of blood, and analyses have also been carried out on stored plasma and on washed cell suspensions. The observed biochemical changes occur only when erythrocytes are present, and they may, therefore, be attributed to changes taking place in these blood cells. The onset of haemolysis in stored blood is associated with an increase in the non-protein nitrogen content of the blood and in the ionic phosphate content of the plasma. A marked increase in the stability of washed cells stored in a sucrose saline solution has been observed. Similar analyses have been carried out on blood stored in the various solutions which have been suggested by other workers. The same biochemical changes are found to take place in these solutions.

Dr. Drevermann has studied cellular changes and the sedimentation rate in blood stored in four different solutions used as preservatives and anti-coagulants, and Miss Splatt and Miss Jakobowicz have carried out analyses for sodium, potassium, magnesium and calcium. Two of the solutions which contained no carbohydrates were found to be of little value because some haemolysis had occurred by the tenth day. In the solution suggested by Maizels and Whittaker, which contains dextrin, and in that of Duran Jorda, containing glucose, haemolysis did not occur until after the fourth week. The biochemical changes indicate that the latter solution is probably more useful, and its value is enhanced by the fact that a smaller volume is added to the shed blood.

The Treatment of Haemorrhage.

Dr. Drevermann has tested two methods of carrying out "arm to arm" transfusions. Of these, the French army method is easier and more effective. The apparatus is small, and is readily transported and sterilised. In collaboration with Major Stanley Williams, he is studying the effects of treatment of shock and haemorrhage by administration of normal human serum, which is being prepared and stored at the Commonwealth Serum Laboratories. Serum presents some advantages for emergency transfusion in shock and after less severe haemorrhage. It can be stored more easily than blood and for more prolonged periods, and by pooling the necessity of typing can be avoided.

For the treatment of anuria following incompatible blood transfusions an attempt has been made to obtain a solution which, injected intravenously in small amounts, will cause rapid alkalinisation of the urine. Sodium bicarbonate is of little value, but a dose of 20 c.c. of molar sodium lactate solution shifts the pH of the urine to the alkaline side and maintains it there for half an hour. It may be possible to produce more significant effects with larger doses or more concentrated solutions.

Vitamin C in the Urine.

Dr. Drevermann has compared the excretion of vitamin C in the urine of patients with peptic ulcer with that of healthy subjects and of patients suffering from other conditions. It was found that the output from patients in the first group was much less than that from persons in the control groups. Patients with conditions other than peptic ulcer were found to have a smaller excretion than normal subjects. A study of the diets of those patients with peptic ulcer, before and after the development of symptoms, indicates that the deficiency is unlikely to be an aetiological factor in peptic ulceration, but the correction of deficiency undoubtedly produces some clinical improvement. That minor deficiencies in vitamin C storage in the body can develop relatively rapidly was shown by the investigation of twenty men of the Australian Militia, who, during the previous month, had been given a diet with a very low content of this vitamin.

Treatment of Malignant Neutropenia with Marrow Extract.

Dr. Drevermann and Dr. Gardner have prepared an extract of yellow bone marrow by saponification with alcoholic potash and extraction with petroleum ether. This extract has been used in the treatment of five cases of malignant neutropenia. In three its administration has been the only method of treatment. One of these patients recovered dramatically, another showed haematological recovery without corresponding clinical improvement, while the third failed to respond. In two cases pentnucleotide was given with the extract. In one, rapid recovery followed, but in the other there was no improvement. Although these results are not so good as those of other observers, they are sufficiently encouraging to warrant the continued use of marrow extracts in the treatment of this condition.

Ian J. Wood:

''The Transfusion of Stored Blood: Some Observations on the Organisation of a Blood Transfusion Service in Times of National Emergency,'' ''Medical Journal of Australia,'' 1939, 2, 768.

Marjorie Bick and Ian J. Wood:

''Some Observations on the Biochemical Changes Associated with Haemorrhage from the Stomach and Duodenum'' (in the press).

''Biochemical Changes in Paralytic Ileus'' (in the press).

Marjorie Bick:

''The Biochemical Changes Occurring During the Storage of Human Blood,'' ''The Australian Journal of Experimental Biology and Medical Science,'' 1939, 17, p. 321.

E. B. Drevermann and Hilda J. Gardner:

''Schultz Syndrome with Special Reference to Treatment with Extract of Yellow Bone Marrow,'' ''Medical Journal of Australia,'' 1940, 1, 851.

E. B. Drevermann:

''Experimental Variation of the Urea Content of the Blood,'' ''Medical Journal of Australia,'' 1939, 2, p. 747.

NEUROPATHOLOGICAL RESEARCH.

Dr. E. Graeme Robertson has continued his pathological studies of material from human cases of nervous disease which have been fully investigated by clinical methods. He has completed his work with Dr. Burnet and Dr. Jackson on experimental material from monkeys infected with the virus of poliomyelitis. The main results of this work were described in last year's report. His chief preoccupation this year has been the study of encephalography and the preparation of a monograph embodying some modifications of the method. He has carefully determined the position of small quantities of air revealed by Roentgengrams taken at various times during its slow injection into the theca. The routes taken have been described and found to depend upon the posture of the patient. When injected by the method described the ventricles can be outlined without filling the general sub-arachnoid space. The anatomical pathway followed by the air to the ventricles is described together with the postures necessary to direct the air into any particular region of the ventricles, sub-arachnoid space, or cisterns. The method allows of the visualisation of the fourth as well as of the third and lateral ventricles. It is even possible to obtain X-ray photographs of the cisterna magna alone and the path followed by the air on its way into the fourth ventricle. The value of encephalography for the localisation of intra-cerebral lesions has been well established by other workers, and Dr. Robertson is recording in the monograph not only his own methods in exact detail, but also the results of a large series of encephalograms which illustrate fine points of diagnosis. The monograph will be profusely illustrated, and in the description of the methods diagrams illustrating the distribution of air in the ventricles and photographs showing the posture of the patient accompany the X-ray pictures.

E. Graeme Robertson:

> ''An Examination of the Olfactory Bulbs in Fatal Cases of Poliomyelitis During the Victorian Epidemic of 1937-1938,'' ''The Medical Journal of Australia,'' 1940, 1, p. 156.

THE BLOOD BANK.

The blood bank, which was established in the Institute last year by Dr. Ian Wood and Miss Williams, has proved thoroughly satisfactory, and supplies the emergency blood transfusion requirements without overtaxing the accommodation in the large refrigerator provided for this purpose by the Red Cross. Since no other blood bank has yet been set up in Melbourne, we have supplied blood, when needed in emergency, not only to the Royal Melbourne Hospital, but to other metropolitan hospitals and to doctors in private practice. We are deeply indebted to the Red Cross Voluntary Donor Service, who have provided the blood for the bank. Blood has also been available for the various studies on stored blood mentioned elsewhere in this report. In transfusing with stored blood there have been no serious reactions. The average duration of storage has been fourteen days, and the maximum period 27 days. The average weekly output has been eight pints. The Red Cross has provided transfusion apparatus since the outbreak of the war, and the remainder of the maintenance of the bank has been partly a charge on the Institute and partly on a research grant from the National Health and Medical Research Council, since a supply of stored blood was demanded for researches being carried out under this grant.

RED CROSS TRANSFUSION SERVICE.

Since its inception in 1929, facilities have been made available at the Institute for the examination of donors for this service. This consists of a general physical examination and the determination of blood group, iso-agglutinin titre of the serum, Wassermann reaction, haemoglobin content of the blood, and tests of the urine. This work is done in the evenings, usually at weekly intervals. Groups of 12-16 volunteers are examined at each session. The honorary medical officer of the service, Dr. Bryce, has had the co-operation of the medical superintendents of the public hospitals (or their deputies), who in rotation carry out the physical examinations, and the voluntary assistance of Miss B. Splatt and Miss R. Jakobowicz.

During the past year 211 new donors have been enrolled. Periodic re-examination of donors who have given more than one transfusion have also been carried out.

The number of transfusions given by Red Cross donors in 1939 was 846. As the total number of calls received since the service began is 3411, these figures illustrate the increasing demands which have been made on the service in recent years. This regular service supplies donors without charge to all the public hospitals for individual transfusions, and also the majority of the donors for the blood bank maintained at this Institute. Since 1938 Red Cross donors have also been available to private practitioners, and the fees received from this source are devoted to the maintenance expenses of the service.

Early in 1939 a scheme was drawn up for an emergency service. This was put into operation in May, 1939. Appeals were made to women and men not liable for military service to enrol, and about 1500 persons responded. The organisation of the emergency service has involved the co-operation of the Red Cross Society, the medical profession, and the community in general, but the necessary laboratory tests and preparation and care of equipment have been carried out almost entirely at the Institute, although help has been received from workers in other laboratories. In the early stages the tests were carried out by Dr. Bryce, with voluntary assistance from Dr. H. Gardner, Dr. March Avery, Miss Mavis Freeman, Miss Phyllis Rountree and others. As the work increased, however, it was considered necessary, in order to carry them out with the necessary expedition, to appoint a half-time officer. Dr. Dorothy Gepp undertook this work from October to December, 1939. It was found, however, to occupy more than half-time, which was the maximum that Dr. Gepp could give. In February, therefore, Dr. R. Jakobowicz was appointed to assist Dr. Bryce on a full-time basis, with the proviso that she could continue to carry out investigations of cases of special interest in connection with the biochemical work in which she had been previously associated with Dr. C. Wallace Ross.

Since the emergency service was started several developments of blood transfusion, not actually visualised at its inception, but directly associated with war-time needs, have taken place. In connection with these developments emergency donors have therefore been asked to serve in the following capacities:—

(1) To supplement the regular donors in maintaining supplies of blood for the blood bank.

(2) To supply blood for the preparation of stored serum, for use in the Army Medical Service overseas.

(3) To supply blood for the preparation of the testing serum necessary for blood group tests.

The emergency service involves a great deal of preparation work in the cleaning of slides and in the cleaning and sterilising of syringes, needles, and tubes. This work has been undertaken by the junior laboratory assistants as a voluntary overtime service.

WAR WORK.

The war has imposed a considerable amount of additional and varied work, principally of a routine nature, upon the staff of the Institute and of the Hospital Routine Services. Dr. F. M. Burnet and other members of the staff have been available for advice on a number of problems. Various disinfectants and other substances have been tested, and Miss Williams investigated a number of internees who were possible carriers of amoebic dysentery. The blood groups of nearly 13,000 troops of the A.I.F. in camp in Victoria were determined here by a team of skilled workers—Major E. V. Keogh, A.I.F.; Dr. Lucy Bryce, Dr. G. V. Rudd, Dr. E. B. Drevermann, Miss Mavis Freeman, Miss F. E. Williams and Miss M. Bick, of the Institute, and Mr. Christie, of the Commonwealth Serum Laboratories, assisted by Miss N. Clarke and Miss Patricia Lind and by several members of the junior staff of the Institute. Captain Colin W. Ross helped greatly in the organisation of this work. A large number of officers, nurses, and masseuses have been vaccinated, inoculated with tetanus prophylactic and T.A.B. and blood grouped by Dr. F. M. Burnet, Dr. Lucy Bryce and Miss Williams.

Transfusion Apparatus for Use in the Australian Navy, Army, and Air Force.

An apparatus for blood transfusion, which has been adopted for use in the Australian Navy, Army, and Air Force, and which provides a container capable of holding two pints of blood, together with the citrate and glucose and necessary additional tubing, needles, etc., for the collection and administration of blood, has been designed. A number of useful modifications of models already in use here and elsewhere have been introduced. Captain Colin W. Ross has been largely responsible for these modifications and for designing the most suitable packing for war conditions. A full description of the apparatus and its use is given in a monograph by a number of authors which will shortly be published.

ROUTINE HOSPITAL SERVICES.

Bacteriology and Clinical Pathology.

The past year again shows an increase in the number of diagnostic tests carried out by this department.

The number of investigations totalled 19,921 for 10,625 patients, and included 2435 Wassermann tests, 209 gonococcal complement fixation tests, and 3850 tests for allergy. Bacteriological and haematological tests comprise the remainder. Therapeutic desensitisation has been carried out in some allergic patients with satisfactory results in most instances. A patient of outstanding interest was one with dermatitis venenata who was found to give positive patch tests to certain cultivated plants, especially Gaillardia and a weed Euphorbia peplus (petty spurge). These results were in conformity with the patient's history. Extracts of these excitants have been prepared in the department, and it is hoped that she will benefit from a course of desensitising injections.

The typing of pneumococci has been continued during the past year. In a patient with septicaemia the infecting pneumococcus (Type XI) was refractory to sulphapyridine therapy in adequate dosage. This raises the question of the advisability of therapeutic sera for types other than the usual Types I and II being available for such cases.

A suitable tissue extract (thromboplastin) was prepared, and some investigations into prothrombin times, especially in cases of jaundice, have been made in order to assess the liability to haemorrhage during operative procedures.

In the haemaotological section of the work many dyscrasias of unusual interest have been encountered. Some of these require repeated and detailed examinations.

In an endeavour to improve the sterilisation of hypodermic syringes in use in the wards, the properties of an antiseptic, para-chlor-meta-xylenol, were investigated. This was found to be a powerful germicide, and will be used throughout the hospital for this purpose as soon as supplies are available. With the co-operation of Mr. C. Macgibbon, the chief dispenser, many improvements have been made in the methods of preparing, storing, and sterilising solutions for hypodermic injection.

Dr. F. M. Kinross was appointed assistant clinical pathologist in succession to Dr. J. D. Hicks. Miss G. E. Morton, M.Sc., was a voluntary assistant in the department for several months, and her energetic co-operation was greatly appreciated. Miss Mavis Freeman, B.Sc., gave voluntary help in the department for some weeks prior to her departure overseas.

BIOCHEMISTRY AND BASAL METABOLISM.

Every year the routine biochemical work of the Hospital is increased by the introduction of new investigations. In the present year the use of sulphanilamide and allied drugs has necessitated numerous estimations of the sulphanilamide concentration in the blood and cerebrospinal fluid. As many patients as possible are examined three and five days after the commencement of treatment to ascertain if an adequate level is reached in the blood by oral or other administration. At the same time the opportunity has been taken of carrying out some special investigations. In collaboration with Mr. Baseley, of the Commonwealth Serum Laboratories, Dr. Jakobowicz and Miss Splatt have made numerous determinations in the blood of horses to determine the dosage required to reach an adequate concentration of the drug. Dr. Stanley Williams, Miss Splatt, and Dr. Jakobowicz have also studied the excretion of sulphanilamide in the saliva in volunteers, to whom the drug was given by Rehfus tube into the stomach, the blood and salivary sulphanilamide being estimated at short intervals for some hours. These data also gave information concerning the rate of absorption of the drug and the dose required to reach an adequate concentration in the blood. The results suggested that a threshold of concentration in the blood had to be reached before excretion in the saliva and indicated that to obtain any prophylactic effect against organisms in the throat large doses would have to be given by the mouth.

Reference has already been made to the work of Miss Splatt and Dr. Jakobowicz on changes in the mineral content of stored blood. Jeanneney and Servanti (1938) had shown that the potassium content of the plasma increases when blood is stored, and Scudder, Drew, Corcoran and Bull (1939) had confirmed these observations and drawn attention to the importance of this pre-haemolytic change. Miss Splatt and Dr. Jakobowicz have followed also the changes in sodium, calcium, magnesium, and chloride. Changes in the concentration of these elements in the plasma were observed as soon as the blood was placed in the anti-coagulant solution and before the onset of haemolysis. The plasma potassium rises rapidly, the plasma sodium and chloride fall, and the calcium and magnesium alter irregularly.

In view of the suggestion that the increased potassium content of the plasma of stored blood is injurious to the recipient, particular attention was paid to this element and to the possibility that sodium may antagonise its action. Analysis of recipient's blood before and after transfusions with stored blood or stored plasma show that although changes in concentration of sodium, calcium and chloride occur, there is no increase in the potassium content of the recipient's serum even if two pints of plasma of high potassium content are injected.

Analyses have been made of blood from a series of normals and from donors after venesection and patients after haemorrhage to investigate the possibility that changes in the distribution of potassium between the blood cells and plasma occur after haemorrhage, as indicated by the work of Schmidt and Basse (1936) and others. Only negative results have so far been obtained.

At the suggestion of Dr. C. Wallace Ross, the Takata-Ara test has been performed by Dr. Jakobowicz on a number of patients with liver disease, and other tests for liver function previously in use were performed at the same time, including the hippuric acid test and the Van den Bergh reaction.

The equipment of the laboratory has been augmented by the generosity of the Royal Melbourne Hospital Auxiliaries. An incubator, electric water-bath with thermostatic control, and a still have proved very useful. The gift of a second Benedict Roth Machine has helped in coping with the increasing number of determinations of Basal Metabolic rate.

P. L. BASELEY, RACHEL JAKOBOWICZ, and BERYL SPLATT: "Dose Rates and Blood Concentrations of Sulphanilamide in Horses," "The Australian Veterinary Journal," 1939, 15, 199.

STANLEY WILLIAMS, BERYL SPLATT, and RACHEL JAKOBOWICZ: "The Concentration of Sulphanilamide in the Saliva Following Oral Administration," "The Medical Journal of Australia," 1940, 1, 120.

ELECTROCARDIOGRAPHY.

During the year 940 electrocardiograms, approximately 75 p.c. of which included lead IV F, have been taken with the "Mathews" portable instrument presented by the ladies of the Box Hill and Mont Albert Auxiliary in 1932.

MORBID ANATOMY.

During the year 464 autopsies were performed. Dr. C. H. Mollison and Dr. Wright Smith were responsible for the examination of 1708 biopsy sections from 1239 specimens. Sections taken from autopsy material numbered 715. Routine examination of the appendix removed at operation was carried out on 365 occasions. The routine photography has been done by Miss Helen Wischusen.

MUSEUM.

New specimens added to the Museum included samples of carcinoma of the bile ducts, lymphosarcoma in the spleen, sarcoma of the uterus, intussusception of ileum, massive pulmonary thrombosis, neurocytoma of the adrenals and aneurysm of the heart.

A series of specimens is being prepared for teaching in the out-patients' department.

Specimens have been used during the year by the College of Surgeons and the College of Physicians for examination purposes.

TEACHING.

Dr. Wright Smith has given the usual course of tutorial lectures in Pathology to fourth year students in December and February. Lecture demonstrations in morbid anatomy have been given twice weekly to the fifth and sixth year students, and lectures in elementary bacteriology have been given to the student nurses in the Hospital. Tuition in clinical pathology was given to fifth year medical students by Dr. H. Gardner.

LIBRARY.

Our thanks for the gifts of journals and books are due to the following:—L'Académie Royale de Médecine de Belgique; The Commonwealth Department of Health; The Council of Scientific and Industrial Research; Miss Danks; Mr. Dobell, F.R.S.; The London Hospital; The Medical Research Council; The Middlesex Hospital Medical School; New York State Department of Health, Division of Laboratories and Research; Department of Pathology, University of Oxford; Rockefeller Institute, New

York; Dr. R. J. Wright Smith; La Société Royale des Sciences Médicales; The South African Institute for Medical Research; The University of Harvard, Department of Tropical Medicine; The University of Pennsylvania, Department of Pathology; The Government Institute for Infectious Diseases of the Tokyo Imperial University; The University of Tomsk.

CHARLES H. KELLAWAY,

Director.

The Walter and Eliza Hall Institute of Research in Pathology and Medicine

FINANCIAL STATEMENT FOR THE YEAR ENDED 30th JUNE, 1940.

INCOME ACCOUNT.

RECEIPTS.

To Balance Brought Forward from 30th June, 1939				£3,729	19	4
,, Trustees W. & E. Hall Trust	£3,200	0	0			
,, University of Melbourne	750	0	0			
,, Felton Bequest Committee	270	0	0			
,, Estate Alfred Edments	100	0	0			
,, Estate Anthony Mackie	100	0	0			
,, Donation	3	3	0			
,, Royal Melbourne Hospital—Refund	446	19	3			
,, Fees Received and Materials Sold	299	17	9			
,, Interest	116	13	10			
,, Sundries	0	7	0			
				5,287	0	10
				£9,017	0	2

EXPENDITURE.

By Salaries and Wages	£4,132	15	2			
,, Materials	287	3	7			
,, Apparatus	18	8	3			
,, Sundries	198	7	6			
,, Publications	104	15	8			
,, Fittings and Equipment	6	12	0			
,, Repairs to Apparatus	10	15	5			
,, Repairs to Buildings	11	17	9			
,, Library Account	23	8	1			
,, Bio-chemical Department	351	9	10			
,, Neurological Department	11	7	9			
				£5,157	1	0
,, Balance—						
Investments	1,875	0	0			
Cash — Agent-General, London	52	7	7			
Secretary's Advance A/c	100	0	0			
Bank of N.S.W.	1,832	11	7			
				3,859	19	2
				£9,017	0	2

THE ELSIE MARION CARTY ACCOUNT.

To Balance Brought Forward from 30th June, 1939		£354 15 3	By Salaries and Wages	£238 6 8	
,, Interest	£167 12 0		,, Materials	13 13 11	£252 0 7
,, Estate E. M. Carty	58 0 9		,, Balance, Bank of N.S.W.		338 8 0
,, Refund Medical Research Council	10 0 7	235 13 4			
		£590 8 7			£590 8 7

BIO-CHEMICAL DEPARTMENT ACCOUNT.

To Interest	£323 10 2	By Salaries and Wages	£675 0 0
,, Transfer from Income Account	351 9 10		
	£675 0 0		£675 0 0

LIBRARY ACCOUNT.

To Interest	£70 10 3	By Books, Journals and Bookbinding	£99 12 4
,, Donation—Miss Danks	5 14 0		
,, Transfer from Income Account	23 8 1		
	£99 12 4		£99 12 4

VIRUS ACCOUNT.

	£	s.	d.		£	s.	d.
To Balance Brought Forward from 30th June, 1939	£1,006	19	10	By Salaries and Wages	£1,515	15	10
,, Commonwealth Government	1,065	0	0	,, Materials	963	0	8
,, E. Alec Cato	1,000	0	0	,, Balance	593	3	4
	£3,071	19	10		£3,071	19	10

FUND FOR NEUROLOGICAL RESEARCH.

	£	s.	d.		£	s.	d.
To Balance Brought Forward from 30th June, 1939	£43	19	2	By Salaries and Wages	£54	2	4
,, Transfer from Income Account	11	7	9	,, Materials	1	4	7
	£55	6	11		£55	6	11

ENDOWMENT FUNDS.

	£	s.	d.		£	s.	d.
To Balance Endowment Fund	£8,612	10	3	By Investments	£14,844	8	10
,, Balance Elsie Marion Carty Fund	4,281	3	6	,, Current Account, Bank of N.S.W.	79	4	11
,, Library Fund	2,030	0	0				
	£14,923	13	9		£14,923	13	9

MEDICAL RESEARCH COUNCIL GRANTS.

To Balance Brought Forward from 30th June, 1939	£1,231 1 4	By Salaries and Wages	£3,091 11 8	
,, Grants	2,707 0 0	,, Materials and Apparatus	632 15 5	
				£3,724 7 1
		,, Balance		213 14 3
	£3,938 1 4			£3,938 1 4

POLIOMYELITIS ACCOUNT.

To Balance Brought Forward from 30th June, 1939	£320 10 2	By Salaries and Wages	£231 17 3
,, Medical Research Council Grants	158 0 0	,, Materials	98 18 0
,, Refund Petty Cash	2 15 2	,, Refund to Medical Research Council	150 10 1
	£481 5 4		£481 5 4

AUDITOR'S CERTIFICATE.

I have to report that I have completed the Audit of the Books and Accounts of the Institute for the period ended 30th June, 1940. I have verified all receipts and have had vouchers produced for all disbursements. All information and explanations required have been given. The Statement is a correct statement of Receipts and Expenditure as revealed by the Books of the Institute.

W. M. JARVIE, F.C.A. (Aust.), Auditor.

18th July, 1940.